

Pre-Treatment Procedures

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.
- 5 ▪ Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

Experimental Design

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.
- 10 ▪ Animals were assigned to the treatment groups shown in Table 1. The dose levels administered were shown in Table 2.

Administration of the Test/Control Articles

Group 1 and 2 Animals

- Method of administration: injection in the left inguinal lymph node.
- 15 Animals were lightly anaesthetized before each administration by an intramuscular injection of ketmine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétquinol, Lure, France).

Group 3

- Route: subcutaneous.
 - Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétquinol, Lure, France).
- 25 Animals were also lightly anaesthetized before each administration by an intramuscular injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 3.

▪ **ELISPOT Analysis**

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFN γ assay was used in order to measure IFN γ production from T lymphocytes obtained from the monkeys in response to gp100 antigens.

10 **Materials and Methods**

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFN γ antibodies/G-Z4 1 mg/mL.

Detection antibodies: MABTECH monoclonal anti-IFN γ antibodies/7-B6-1-
15 biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjugate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate
kit/ref: 170-64 32.

Coating

20 Place 100 μ L per well of capture antibodies at 1 μ g/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

Saturation

Place 200 μ L per well of RPMI supplemented with 10% FCS, non essential
25 amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

Test

Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific

stimulus (PMA-Iono). The pooled peptides used in this Example to stimulate IFN- γ production were derived from gp100 and are illustrated in Tables 4 to 7. The final volume of each sample is 200 μ L. Incubate 20 hours at 37°C.

- 5 Wash 4 times in 1X PBS and 0.05% Tween 20.

Detection

Place 100 μ L per well of detection antibodies at 1 μ g/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

- 10 **Reaction**

Place 100 μ L per well of Extravidin-PA conjugate diluted 1/5000 in 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

Substrate Addition

- 15 Place 100 μ L per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each
20 spot corresponds to an individual IFN- γ secreting T cell.

Results

- The animals that tested positive on the ELISPOT analysis are shown in Figures 1-4. Overall, the results demonstrate that of the animals tested, 2
25 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

ELISA Analysis

The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5ug/well. Plates were placed at 4°C overnight. Plates were then washed and blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2) was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H₂O₂ in substrate buffer (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450nm.

Results

The results of the above experiment are presented in Table 8 and in Figure 5. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous

injection of the ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 5, intranodal injection of the antigens induced a humoral response that was much greater than when the antigen
5 was injected subcutaneously.

In summary, the results of this Example demonstrate that intranodal injection of a tumor antigen induces both a humoral and cell mediated response that is much greater than when the tumor antigen is injected by the conventional subcutaneous route of administration.

10 While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the
15 appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

TABLE 1

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	ALVAC(2) - gp100 mod. days 28, 42, 56 *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod. days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

*209(2M)-IMQVPFSY; 29D(9V) YLEPGPVTY

- 5
- Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
 - Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

36
TABLE 2

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: $0.25 \times 10^{7.3}$ CCID 50 ALVAC (2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID50	0.250
	Dose: 200 μ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTY (290(9V)) (100 μ g each)	0.2
3	Saline (NaCl 0.9%)	0.250
	ALVAC(2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID 50	0.250
	Dose: 200 μ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTY (290(9V)) (100 μ g each)	0.2

TABLE 3

Days	Sites used
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

TABLE 4

Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGAIK	SEQ.ID.NO.3
1330	GALLAVGATKVP RNQ	SEQ.ID.NO.4
1331	VGATKVP RNQDWLG V	SEQ.ID.NO.5
1332	VPRNQDWLGVSRLR	SEQ.ID.NO.6
1333	DWLGVSRLRTKAWN	SEQ.ID.NO.7
1334	SRQLRTKAWNRLYP	SEQ.ID.NO.8
1335	TKAWNRLYPEWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQLDC	SEQ.ID.NO.10
1337	EWTEAQLDCWRGGO	SEQ.ID.NO.11
1338	QLDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNDGP	SEQ.ID.NO.13
1340	VSLKVSNDGPTLIGA	SEQ.ID.NO.14
1344	IALNFPGSQKVL PDG	SEQ.ID.NO.15
1345	PGSQKVL PDGQVIWV	SEQ.ID.NO.16
1346	VLPDGQVIWVNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNTHINGSQVWGGQP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGGQPVYPQETDDAC	SEQ.ID.NO.21
1351	VYPQETDDACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGWSQ	SEQ.ID.NO.24
1355	GSWSQKRSFVYVWKT	SEQ.ID.NO.25
1356	KRSFVYVWKTWQGYW	SEQ.ID.NO.26
1357	YVWKTWQGYWQVLGG	SEQ.ID.NO.27
1358	WQGYWQVLGGPVSGL	SEQ.ID.NO.28
1359	QVLGGPVSGLSIGTG	SEQ.ID.NO.29

39
TABLE 5

Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHME	SEQ.ID.NO.31
1362	RAMLGTHMEVTVYH	SEQ.ID.NO.32
1363	THTMEVTVYHRRGSR	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSSS	SEQ.ID.NO.35
1366	SYVPLAHSSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPFVSVS	SEQ.ID.NO.37
1369	DQVPFVSVSQRLAL	SEQ.ID.NO.38
1370	SVSVSQRLALDGGNK	SEQ.ID.NO.39
1372	DGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPSGY	SEQ.ID.NO.42
1375	ALQLHDPSGYLAED	SEQ.ID.NO.43
1379	DFGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVIHTY	SEQ.ID.NO.45
1381	SRALVVTHTYLEPGP	SEQ.ID.NO.46
1382	VTHTYLEPGPVTAQV	SEQ.ID.NO.47
1383	LEPGPVTAQVVLQAA	SEQ.ID.NO.48
1384	VTQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVPGTT	SEQ.ID.NO.51
1388	VPGTTDGHRTAEAP	SEQ.ID.NO.52
1389	DGHRPTAEAPNTTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTEVVGTTPGAQ	SEQ.ID.NO.55
1393	EVVGTTPGAQPTAEP	SEQ.ID.NO.56

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TABLE 6**Peptide Pool #3**

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSTGTS	SEQ.ID.NO.57
1395	PTAEPSTGTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VOVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAPVQMPATAE	SEQ.ID.NO.61
1399	TAPVQMPATAEPTGMT	SEQ.ID.NO.62
1400	MPTAESTGMTPEKVP	SEQ.ID.NO.63
1401	STGMTPEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGMTPAEV	SEQ.ID.NO.68
1408	SIVVLSTGTTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TFARELPIPEPEGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPLLDG	SEQ.ID.NO.77
1417	TGSLGPLLDGTATLR	SEQ.ID.NO.78
1418	PLLDGTATLRLVKRQ	SEQ.ID.NO.79
1419	TATLRLVKRQVPLDC	SEQ.ID.NO.80
1420	LVKRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGFSFV	SEQ.ID.NO.82
1422	VLYRYGFSFVTLDIV	SEQ.ID.NO.83

41
Table 7

Peptide Pool #4

Peptide	Sequence	SEQ.ID.NO.
1424	TLDIVQGIESAELQ	SEQ.ID.NO.84
1425	QGIESAELQAVPSG	SEQ.ID.NO.85
1426	AELQAVPSGEGDAF	SEQ.ID.NO.86
1427	AVPSGEGDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGI	SEQ.ID.NO.88
1429	ELTVSCQGGIPKEAC	SEQ.ID.NO.89
1430	CQGGIPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQRL	SEQ.ID.NO.92
1434	PAORLCQPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGGSGTY	SEQ.ID.NO.96
1441	LADTNLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPQOE	SEQ.ID.NO.98
1443	STQLIMPQOEAGLGQ	SEQ.ID.NO.99
1444	MPGQEAGLGQVPLIV	SEQ.ID.NO.100
1445	AGLGQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVLASLIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPOL	SEQ.ID.NO.103
1451	MKQDFSVPOLPHSSS	SEQ.ID.NO.104
1452	SVPOLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSCPIG	SEQ.ID.NO.107
1455	PRIFCSCPIGENSPL	SEQ.ID.NO.108

TABLE 8

Monkey #	DAY (mOD/min)			
	0	57	68	96
1	3	5	2	2
2	4	6	12	10
3	7	6	10	8
4	7	6	8	8
5	5	9	20	15
6	11	8	10	12
7	11	23	51	30
8	7	30	70	22
9	1	7	5	3
10	2	6	6	4
11	3	7	14	8
12	6	9	15	6

We claim:

1. A method for inducing an immune response in an animal to a tumor
5 antigen comprising administering an effective amount of a tumor
antigen or a nucleic acid sequence encoding a tumor antigen to a
lymphatic site in the animal.
2. A method according to claim 1 wherein the tumor antigen is selected
10 from the group consisting of CEA, gp100, the MAGE family of proteins,
DAGE, GAGE, RAGE, NY-ESO 1, Melan-A/MART 1, TRP-1, TRP-2,
tyrosinase, HER-2/neu, MUC-1, p53, KSA, PSA, PSMA, and fragments
and modified versions thereof.
- 15 3. A method according to claim 1 or 2 wherein the lymphatic site is a
lymph node.
4. A method according to any one of claims 1 to 3 wherein the nucleic
acid is selected from the group consisting of viral nucleic acid,
20 bacterial DNA, plasmid DNA, naked/free DNA, and RNA.
5. A method according to claim 4 wherein the viral nucleic acid is
selected from the group consisting of adenoviral, alphaviral and
poxviral nucleic acid.
25
6. A method according to claim 5 wherein the poxviral nucleic acid is
selected from the group consisting of avipox, orthopox and suipox
nucleic acid.
- 30 7. A method according to claim 5 wherein the poxviral nucleic acid is
selected from the group consisting of vaccinia, fowl pox, canarypox
and swinepox nucleic acid.

8. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC nucleic acid.
- 5 9. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a vector.
- 10 10. A method according to claim 9 wherein the vector is a recombinant virus or bacteria.
11. A method according to claim 10 wherein the recombinant virus is selected from the group consisting of adenovirus, alphavirus and poxvirus.
- 15 12. A method according to claim 11 wherein the poxvirus is selected from the group consisting of avipox, orthopox and suipox.
13. A method according to claim 11 wherein the poxvirus is selected from the group consisting of vaccinia, fowlpox, canarypox and swinepox.
- 20 14. A method according to claim 11 wherein the poxvirus is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC.
- 25 15. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a cell.
16. A method according to any one of claims 1 to 14 wherein the tumor antigen or nucleic acid coding therefor is contained in a vaccine.

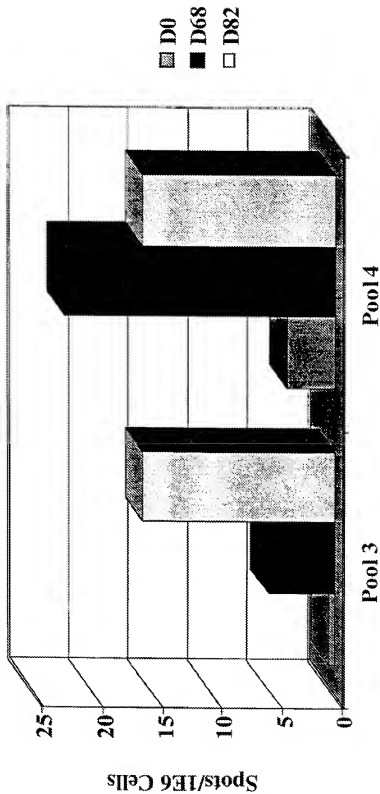
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17. A method according to any one of claims 1 to 16 wherein the tumor antigen is gp100, CEA or a fragment or modified version of gp100 or CEA.
- 5 18. A method according to claim 17 wherein the modified gp100 comprises the sequence IMDQVPFSY (SEQ ID NO: 1) and/or YLEPGPVTY (SEQ ID NO:2).
- 10 19. A method according to claim 17 wherein the modified CEA comprises the sequence shown in Figure 8 (SEQ ID NO:112) and/or YLSGADLNL (SEQ ID NO:113).

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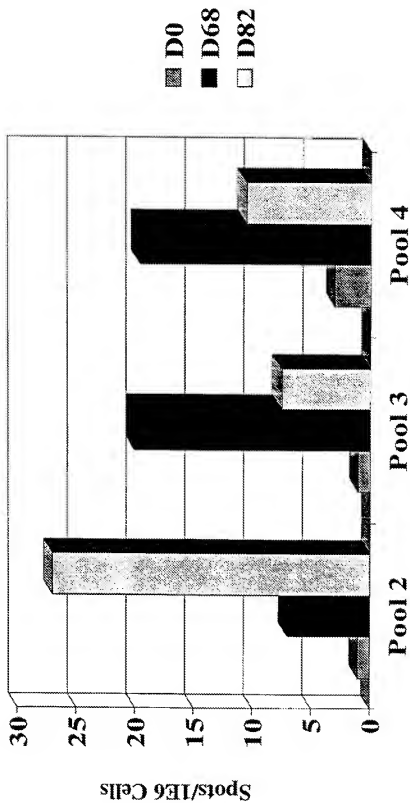
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FIGURE 1
Monkey #6 (Intranodal Administration)



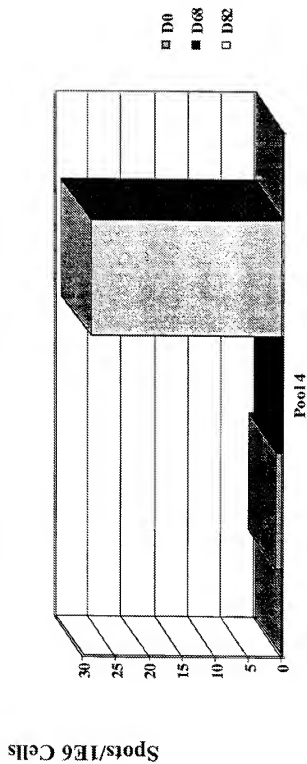
2/11

FIGURE 2
Monkey #7 (Intranodal Administration)



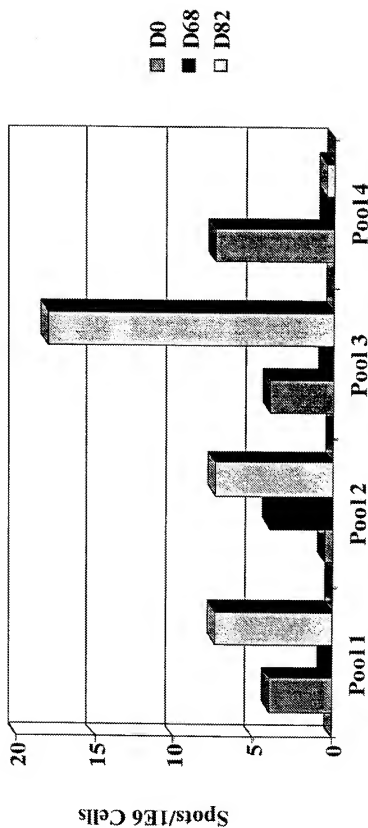
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FIGURE 3
Monkey # 11 (Subcutaneous Administration)



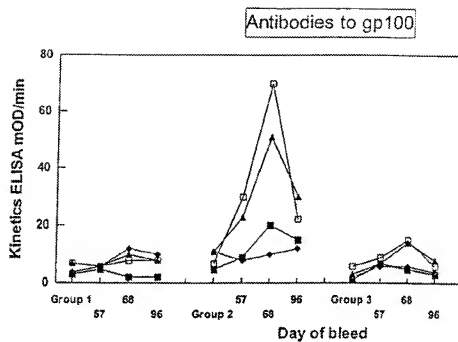
4/11

FIGURE 4
Monkey #10 (Subcutaneous Administration)



5/11

FIGURE 5



6/11

FIGURE 6

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      ATGG  ATCTGGTGCT  AAAAAAGATGC  CTTCTTCATT  TGGCTGTGAT
AGGTGCTTTG  CTGGCTGTGG  GGGCTACAAA  AGTACCAGGA  AACCAGGACT  GGCFTGGTGT
CTCAAGGCCAA  CTCAGAAACCA  AAGCCTGGAA  CAGGCAGCTG  TATCCAGAGT  GGCAGAGAAGC
CCAGAGACTT  GACTCCTGGA  GAGGTGGTCA  AGTGTCCCTC  AAGGTCAGTA  ATGATGGGCC
TACACTGATT  GGTGCAAATG  CCTCCTTCTC  TATTGCCCTG  AACTTCCCTG  GAAGCCAAAA
GGTATTGCCA  GATGGGCAGG  TTATCTGGGT  CAACAATACC  ATCATCAATG  GGAAGCCAGGT
GTGGGGAGGA  CAGCCAGTGT  ATCCCCAGGA  AACTGACGAT  GCCTGCATCT  TCCCTGATGG
TGGACCTTGC  CCATCTGGCT  CTTGGTCTCA  GAAGAGAAGC  TTTGTTTATG  TCTGGAAGAC
CTGGGGCCAA  TACTGGCAAG  TTCTAGGGGG  CCCAGTGTCT  GGGCTGAGCA  TTGGGACAGG
CAGGGCAATG  CTGGGCACAC  ACACGATGGA  AGTGACTGTC  TACCATCGCC  GGGGATCCCG
GAGCTATGTG  CCTCTTGCTC  ATTCCAGCTC  AGCCTTCACC  ATTATGGACC  AGGTGCCTTT
CTCCGTGAGC  GTGTCCCACT  TGGGGGCCCT  GGATGGAGGG  AACAAGCACT  TCCTGAGAAA
TCAGCCTCTG  ACCTTTGCC  TCCAGCTCCA  TGACCCCACT  GGCCTATCTGG  CTGAAGCTGA
CCTCTCCTAC  ACCTGGGACT  TTGGAGACAG  TAGTGGAAAC  CTGATCTCTC  GGGCACTTGT
GGTCACTCAT  ACTTACCTGG  AGCCTGGCCC  AGTCACTGTT  CAGGTGGTCC  TGCAGGCTGC
CATTCCTCTC  ACCTCCTGTG  GCTCCTCCCC  AGTTCAGGC  ACCACAGATG  GGCACAGGCC
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TACACCTGGT  CAGGCGCCAA  CTGAGAGGCC  CTCTGGAACC  ACATCTGTGC  AGGTGCCAAC
CACTGAAGTC  ATAAGCACTG  CACCTGTGCA  GATGCCAACT  GCAGAGAGCA  CAGGTATGAC
ACCTGAGAAG  GTGCCAGTTT  CAGAGGTCT  GGGTACCACA  CTGCGAGAGA  TGTCAACTCC
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TGCACAGTAG  ACAACTACAG  AGTGGGTGGA  GACCACAGCT  AGAGAGCTAC  CTATCCCTGA
GCCTGAAGGT  CCAGATGCCA  GCTCAATCAT  GTCTACGGAA  AGTATTACAG  GTTCCCTGGG
CCCCCTGCTG  GATGGTACAG  CCACCTTAAG  GCTGGTGAAG  AGACAAGTCC  CCCTGGATTG
TGTTCTGTAT  CGATATGGTT  CCTTTCCGT  CACCTGGAC  ATTGTCCAGG  GTATTGAAAG
TGCCGAGATC  CTGCAAGCTG  TGCCGTCCGG  TGAGGGGGAT  GCATTTGAGC  TGACTGTGTC
CTGCCAAGGC  GGGCTGCCCA  AGGAAGCCTG  CATGGAGATC  TCATCGCCAG  GGTGCCAGCC
CCCTGCCAG  CAGGTGTGCC  AGCCTGTGCT  ACCCAGCCCA  GCCTGCCAGC  TGGTTCTGCA
CCAGATACTG  AAGGGTGGCT  CGGGGACATA  CTGCCCTAAT  GTGTCTCTGG  CTGATACCAA
CAGCCTGGCA  GTGGTCAGCA  CCCAGCTTAT  CATGCCTGGT  CAAGAAGCAG  GCCTTGGGCA
GGTCCCGTGT  ATCGTGGGCA  TCTTGCTGGT  GTTGATGGCT  GTGGTCCTTG  CATCTCTGAT
ATATAGGCGC  AGACTTATGA  AGCAAGACTT  CTCCGTACCC  CAGTTGCCAC  ATAGCAGCAG
TCACTGGCTG  CGTCTACCCC  GCATCTTCTG  CTCTTGTCCT  ATTGGTGAGA  ACAGCCCCCT
CTCAGTGGG  CAGCAGGTCT  GA

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7/11

FIGURE 7

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Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
  1           5           10           15
Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
  20           25           30
Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
  35           40           45
Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
  50           55           60
Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
  65           70           75           80
Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
  85           90           95
Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
  100          105          110
Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
  115          120          125
Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
  130          135          140
Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
  145          150          155          160
Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
  165          170          175
Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
  180          185          190
Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
  195          200          205
Ile Met Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
  210          215          220
Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
  225          230          235          240
Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
  245          250          255
Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
  260          265          270
Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Val
  275          280          285
Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
  290          295          300
Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
  305          310          315          320
Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
  325          330          335
Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
  340          345          350
Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
  355          360          365

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8/11

FIGURE 7 (CONT'D)

Ala	Glu	Ser	Thr	Gly	Met	Thr	Pro	Glu	Lys	Val	Pro	Val	Ser	Glu	Val
370						375					380				
Met	Gly	Thr	Thr	Leu	Ala	Glu	Met	Ser	Thr	Pro	Glu	Ala	Thr	Gly	Met
385					390					395				400	
Thr	Pro	Ala	Glu	Val	Ser	Ile	Val	Val	Leu	Ser	Gly	Thr	Thr	Ala	Ala
				405				410						415	
Gln	Val	Thr	Thr	Thr	Glu	Trp	Val	Glu	Thr	Thr	Ala	Arg	Glu	Leu	Pro
				420				425					430		
Ile	Pro	Glu	Pro	Glu	Gly	Pro	Asp	Ala	Ser	Ser	Ile	Met	Ser	Thr	Glu
		435				440					445				
Ser	Ile	Thr	Gly	Ser	Leu	Gly	Pro	Leu	Leu	Asp	Gly	Thr	Ala	Thr	Leu
450					455						460				
Arg	Leu	Val	Lys	Arg	Gln	Val	Pro	Leu	Asp	Cys	Val	Leu	Tyr	Arg	Tyr
465					470					475				480	
Gly	Ser	Phe	Ser	Val	Thr	Leu	Asp	Ile	Val	Gln	Gly	Ile	Glu	Ser	Ala
				485				490						495	
Glu	Ile	Leu	Gln	Ala	Val	Pro	Ser	Gly	Glu	Gly	Asp	Ala	Phe	Glu	Leu
		500						505					510		
Thr	Val	Ser	Cys	Gln	Gly	Gly	Leu	Pro	Lys	Glu	Ala	Cys	Met	Glu	Ile
		515					520					525			
Ser	Ser	Pro	Gly	Cys	Gln	Pro	Pro	Ala	Gln	Arg	Leu	Cys	Gln	Pro	Val
530					535						540				
Leu	Pro	Ser	Pro	Ala	Cys	Gln	Leu	Val	Leu	His	Gln	Ile	Leu	Lys	Gly
545					550					555				560	
Gly	Ser	Gly	Thr	Tyr	Cys	Leu	Asn	Val	Ser	Leu	Ala	Asp	Thr	Asn	Ser
				565				570						575	
Leu	Ala	Val	Val	Ser	Thr	Gln	Leu	Ile	Met	Pro	Gly	Gln	Glu	Ala	Gly
				580				585						590	
Leu	Gly	Gln	Val	Pro	Leu	Ile	Val	Gly	Ile	Leu	Leu	Val	Leu	Met	Ala
		595				600						605			
Val	Val	Leu	Ala	Ser	Leu	Ile	Tyr	Arg	Arg	Arg	Leu	Met	Lys	Gln	Asp
		610				615					620				
Phe	Ser	Val	Pro	Gln	Leu	Pro	His	Ser	Ser	Ser	His	Trp	Leu	Arg	Leu
625					630					635				640	
Pro	Arg	Ile	Phe	Cys	Ser	Cys	Pro	Ile	Gly	Glu	Asn	Ser	Pro	Leu	Leu
				645				650						655	
Ser	Gly	Gln	Gln	Val											
				660											

9/11

FIGURE 8

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ATGGAGTCTCCCTCGGGCCCTCCCCACAGATGGTGCATCCCTGGCAGAGGCTCCTGCTC
1 -----+----- 60
TACCTCAGAGGGAGCGGGGAGGGGTCTTACCACGTAGGGGACCGCTCCGAGGACGAG
a M E S P S A P P H R W C I P W Q R L L L ~
ACAGCCTCAGCTTCTAACTTTTGGAAACCGGCCACCACTGCCAAGCTCAGTATTGAATCC
61 -----+----- 120
TGTCGAGTGAAGATTGGAAGACCTTGGCGGGGTGGTGACGGTTGAGTGTAACTTAGG
a T A S L L T F W N P P T T A K L T I E S ~
ACGCGTTCAATGTCGACAGGGGAGGAGGTGCTTCTACTTGTCCACAATCTGCCCCAG
121 -----+----- 180
TGGCGCAGTTACAGGCTCTCCCTTCTCTCCAGGAGATGACAGGTGTTAGACGGGTC
a T P F N V A E G K E V L L L V H N L P Q ~
CATCTTTTGGCTACAGCTGGGTACAAGGTGAAAGAGTGGATGGCAACCGTCAAATTATA
181 -----+----- 240
GTAGAAAACCGATGTCAGACCATGTTCCACTTTCTCACTACCGTTGGCAGTTAATAT
a H L F G Y S W Y K G E R V D G N R Q I I ~
GGATATGTAATAGGAATCAACAGCTACCCAGGGGCCCATACAGTGGTCGAGAGATA
241 -----+----- 300
CCTATACATTAATCTTGAAGTGTTCGATGGGGTCCGGCGGTATGTCACAGGCTCTCAT
a G Y V I G T Q Q A T P G P A Y S G R E I ~
ATATACCCCAATGCATCCCTGCTGATCCAGAACATCATCCAGATGACACAGGNTTCTAC
301 -----+----- 360
TATATGGGGTTACGTAGGGACGACTAGGTCTTGTATAGGTCTTACTGTGCTCAAGATG
a I Y P N A S L L I Q N I I Q N D T G F Y ~
ACCCCTACAGCTCATAAAGTCAGATCTTGTGAATGAAGAGCAACTGGCCAGTTCGGGTA
361 -----+----- 420
TGGGATGTGAGTATTTAGTCTAGAACCTTACTTCTGCTTGACCGGTCAAGGCCCAT
a T L H V I N S D L V N E E A T G Q F R V ~
TACCGAGAGTGGCCAGGCCCTCCATCTCCAGCAACCACTCCAAACCGGTGGAGGACAG
421 -----+----- 480
ATGGGCTCTAGCGGTTCCGGAGGTAGAGTGGTGTGTGAGGTTGGGACCTCTCGTTTC
a Y P E L P K P S I S S N N S K P V E D K ~
GATGCTGTGGCTTCACTGTGGAACCTGAGACTCAGGACGCAACCTACCTGTGTGGGTA
481 -----+----- 540
CTACGACACGAGTGGACACTTGGACTCTGAGTCTCGCTTGGATGGACACCAACCAT
a D A V A F T C E P E T G D A Y Y L W W V ~
ANCAATCAGAGCCTCCGGGTAGTCCCGAGGCACAGCTGTCCAATGGCAACAGGACCTC
541 -----+----- 600
TTGTTAGTCTCGGAGGGCCAGTCAAGGTCCGACGTCGACAGGTTACCGTTGTCTGGGAG
a N N Q S L P V S P R L Q L S N G N R T L ~
ACTCTATTAAATGTCACAGAAATGACACAGCAAGCTCAAAATGTGAACCCGAGACCCA
601 -----+----- 660
TGAGATAAGTTACACTCTTCTTTACTGTGTGTTGATGTTTACACTTTGGGCTTGGGT
a T L F N V T R N D T A S Y K C E T Q N P ~
GTAGTGGCAGGCCAGTGATTCAGTCATCTGATGTCTCTATGGCCGAGTGGCCCC
661 -----+----- 720
CACTCACUSTCCGCGTCACTAAGTCAGTAGGACTTACAGGAGATACCGGGCTACGGGGG
a V S A R R S D S V I L N V L Y G P D A F ~

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10/11

FIGURE 8 (CONT'D)

ACCATTTCCTCTAAACACATCTTACAGATCAGGGGAAATCTGAACCTCTCTGCCAC
 721 TGGTAAAGGGAGATTITGTGTAATGTCFAGTCCCTTTAGACTTGGAGAGCACGGTG 780
 a T I S P L N T S Y R S G E H L N L S C H -
 GCAGCCTCTAACCCACCTGCACAGTACTCTTGGTTTGTCAATGGGACTTTCAGCAATCC
 781 CGTCGGAGATTGGGTGGACGTCTCATGAGACCAACAGTTACCCCTGAAGGTCTGTAGG 840
 a A A S N P P A Q Y S W F V N G T F Q Q S -
 ACCCAAGAGCTCTTTATCCCAACATCATCTGTGAATAATATGATGATCCTATACCTGCCAA
 841 TGGGTCTTCGAGAAATAGGGTCTAGTGACACTTATATCACCTAGGATATGCCAGGT 900
 a T Q E L F I P N I T V N N S G S Y T C Q -
 GCCCATACTCAGACACTGGCCTCAATAGGACACAGTCCAGACGATCAGAGTCTATGAG
 901 CGGGTATTGAGTCTGTGACCGGAGTTATCTGGTGTCACTGCTGCTAGTGTGAGATATC 960
 a A H N S D T G L N R T T V T T I T V Y E -
 CCACCAAAACCTTCTATCACCAGCACAACTCCAAACCCCTGAGAGATGAGGATGCTGTA
 961 GGTGGGTTGGGAAGTAGTGGTCTGTGTGAGGTGGGGCACTCTCTACTCTACGACAT 1020
 a P P K K F F I T S N N S N P V E D E D A V -
 GGCCTAACCTGTGAACCTGAGATTCAAGACACAACCTTACCTGTGGTGGGTAATATACAG
 1021 CGGAATTGAGACTCTGGACTCTAAGTCTTGTGTTGAGTGACACCAACCCATTTATATGTC 1080
 a A L T C E F E I Q N T T Y L N W V N N Q -
 AGCCTCCCGGTCACTCCAGGCTGAGCTGTCCAATGACACAGGACCTCACTCTACTC
 1081 TCGGAGGGCCAGTCAGGCTCGACGTCGACAGGTTACTGTTCTCTGGGAGTGAGATGAG 1140
 a S L P V S P R L Q L S N D N R T L T L L L -
 AGTGTCACAGGAATGATGTAGGACCTATGAGTGTGGAATCCAGAACGAATTAGTGTT
 1141 TCACAGTGTCTTACTACATCTCTGGGATACTCACACCTTAGTGCTTGTCTAATTCACAA 1200
 a S V T R N D V G P Y E C G I Q N E L S V -
 GACCACAGCGACCCAGTCATCCTGAATGTCTGTATGGCCCGACACACCCACCATTTCC
 1201 CTGGTGTGCTGGTCACTAGGACTTACAGGAGATACCGGCTCTGCTGGGAGTGAAGG 1260
 a D H S D P V I L N V L Y G P D P T I S -
 CCCTCATACACCTATTACCGTCCAGGGGTGAACCTCAGCCTCTCTGCCATGCAGCCTCT
 1261 GGGAGTATGGGATAATGACAGGTCCCLACTTGGAGTGGAGAGGACGGTACCTCGGAGA 1320
 a P S Y T Y Y R P G V N L S L S C H A A S -
 AACCCACCTGCACAGTATCTTGGCTGATTGATGGGAACATCCAGCAACACACACAGAG
 1321 TTGGTGGACGTGTCTAAGAACCGACTTACTACCTTGTAGGTCTGTGTGTGTGTCTC 1380
 a N P P A Q Y S W L I D G N I Q Q H T Q E -
 CTTCTTATCTCCAACATCACTGAGAGAGACAGCGGACTCTATACCTGGCAGGCCATAC
 1381 GAGAAATAGAGGTGTAGTGACTCTCTCTCGGCTGAGATATGACAGCTCGGCTTATGG 1440
 a L F I S N I T E K N S G L Y T C Q A N N -

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11/11

FIGURE 8 (CONT'D)

1441 TCAGCCAGTGGCCACAGCAGGACTACAGTCAAGACAATCACAGTCTCTCGGAGGCTGCC

 AGTGGTGCACCGGTGCGTCTTGATGTCAGTCTGTTAGTGTGACAGACCGCTCGAGGGG 1500
 a S A S G H S R T T Y V K T I T V S A E L P -

 1501 AAGCCCTCCATCTCCAGCAACCACTCCAAACCCGTGGAGGCAAGGATGCTGTGGCCTTC

 TTCCGGAGGTAGAGGTCGTGTGAGGTTTGGGCACCTCCTGTTCCTAGACACCGGAG 1560
 a K P S I S S N N S K P V E D K D A V A F -

 1561 ACCTGTGAACTCGAGGCTCAGAACACACCTTACCTGTGGTGGTAAATGGTCAGAGCCTC

 TGGACACTTGGACTCCGAGTCTGTGTGTGGATGGACACCAACCAITTACCAGTCTCGGAG 1620
 a T C E F E A Q N T T Y L W W V N G Q S L -

 1621 CCAGTCAGTCCAGGCTCCAGCTGTCCAATGGCAACAGGACCCCTCACTCTATTCAATGTC

 GGTCAAGTCAGGTCGGACGTGCACAGGTACCGTTGTCTCGGAGTGAGTAAGTACAG 1680
 a P V S F R L Q L S N G N R T L T L F N V -

 1681 ACAGAAATGACGCAAGAGCCTATGTATGTGGAAATCCAGAACTCACTGAGTGCAAACCGC

 TGTTCCTTACTGCGTTCTCGGATACATACACCTTAGGCTTGAGTCACTCACGCTTGGGG 1740
 a T R N D A R A Y V C G I Q N S V S A N R -

 1741 AGTGACCCAGTCAACCTGGATGTCCTCTATGGGCGGGACACCCCAATCAFTTCCCCCCCC

 TCACTGGGTCACTGGGACCTACAGGAGATACCGGCTCTGGGGGTAGTAAAGGGGGGGT 1800
 a S D F V T L D V L Y G P D T P I I S F P -

 1801 GACTCGTCTTACCTTTGGGAGCGGACCTCAACCTCTCTGCGCACTGGGCTCTAACCCTA

 CTGAGCAGAATGGAAGCCCTCGCTGGAGTTGGAGAGGACGCTGAGCCGGAGATTGGGT 1860
 a D R S Y L S G A D L N L S C H S A S N F -

 1861 TCCCCGCACTATTCTTGGCGTATCAATGGGATACCGCAGCACACACAAAGTTCTCTTT

 AGGGGCGCTATAGAAACCGCATAGTTACCTATGGCGTCTGTTGTGTTCAGAGAGAA 1920
 a S P Q Y S W R I N G I P Q Q H T Q V L F -

 1921 ATGCGCAAAATCAGCGCAATATAACGGGACCTATGCTCTTTGTTGTCTCAACTTGGCT

 TAGCGGTTTAGTGGCGTTTATTATGCGCTGGATACGACAAACAGAGATTGAAACGA 1980
 a I A K I T P N N N G T Y A C F V S N L A -

 1981 ACTGGCCCAATAATTCATAGTCAAGAGCATCACAGTCTTGTGATCTGGAATCTCTCT

 TGACCGCGCTATTAAAGGTATCAGTCTCTGTAGTGTGAGAGAGTATAGACCTTGAAGAGGA 2040
 a T G R H N S I V K S I T V S A S G T S F -

 2041 GGTCTCTCAGCTGGGGCCACTGTGCGCATCATGATTGGAGTGTCTGTTGGGTTGTCTIG

 CCAGAGAGTGAGCCCGGTGACAGCGGTAGTACTAACCTCAGGACCAACCCCAACGAGAC 2100
 a G L S A G A T V G I M I G V L V G V A L -

 2101 ATATAG ←
 ----- 2106
 TATATC
 a I ←

INTERNATIONAL SEARCH REPORT

In ternational Application No

PCT/CA 00/01253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, SCISEARCH, EPO-Internal, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47271 A (GUO YAJUN) 18 December 1997 (1997-12-18) page 23, line 14 -page 24, line 22	1-3,15, 16
X	RAO V S ET AL: "PARTIAL CHARACTERIZATION OF TWO SUBPOPULATIONS OF T-4 CELLS INDUCED BY ACTIVE SPECIFIC INTRALYMPHATIC IMMUNOTHERAPY IN MELANOMA PATIENTS" PROCEEDINGS AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 27, 1986, page 325 XP000990377 ISSN: 0197-016X the whole document	1,2,16

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16 March 2001

Date of mailing of the international search report

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Int. Patent Application No.
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C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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